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Antimicrobial Resistance in Urinary Tract Pathogens in Canada from 2007 to 2009: CANWARD Surveillance Study▽

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From January 2007 to December 2009, an annual Canadian national surveillance study (CANWARD) tested 2,943 urinary culture pathogens for antimicrobial susceptibilities according to Clinical and Laboratory Standards Institute guidelines. The most frequently isolated urinary pathogens were as follows (number of isolates, percentage of all isolates): *Escherichia coli* (1,581, 54%), enterococci (410, 14%), *Klebsiella pneumoniae* (274, 9%), *Proteus mirabilis* (122, 4%), *Pseudomonas aeruginosa* (100, 3%), and *Staphylococcus aureus* (80, 3%). The rates of susceptibility to trimethoprim-sulfamethoxazole (SXT) were 78, 86, 84, and 93%, respectively, for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. aureus*. The rates of susceptibility to nitrofurantoin were 96, 97, 33, and 100%, respectively, for *E. coli*, enterococci, *K. pneumoniae*, and *S. aureus*. The rates of susceptibility to ciprofloxacin were 81, 40, 86, 81, 66, and 41%, respectively, for *E. coli*, enterococci, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*. Statistical analysis of resistance rates (resistant plus intermediate isolates) by year for *E. coli* over the 3-year study period demonstrated that increased resistance rates occurred only for amoxicillin-clavulanate (from 1.8 to 6.6%; $P < 0.001$) and for SXT (from 18.6 to 24.3%; $P = 0.02$). For isolates of *E. coli*, in a multivariate logistic regression model, hospital location was independently associated with resistance to ciprofloxacin ($P = 0.026$) with higher rates of resistance observed in inpatient areas (medical, surgical, and intensive care unit wards). Increased age was also associated with resistance to ciprofloxacin ($P < 0.001$) and with resistance to two or more commonly prescribed oral agents (amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and SXT) ($P = 0.005$). We conclude that frequently prescribed empirical agents for urinary tract infections, such as SXT and ciprofloxacin, demonstrate lowered *in vitro* susceptibilities when tested against recent clinical isolates.

There are an estimated 150 million urinary tract infections per year worldwide (31). In the United States, urinary tract infections result in approximately 8 million physician visits per year (33); they are the most common bacterial infections in women, and account for significant morbidity and associated health care costs (10, 31). Most visits to physicians for symptoms of acute cystitis do not result in urine culture or isolate antimicrobial susceptibility testing. Rather, urine culture is often reserved for patients failing empirical therapy and those with recurrent or complicated infections. Among both outpatients and inpatients, *Escherichia coli* is the primary urinary tract pathogen. It is estimated to account for 75 to 90% of uncomplicated urinary tract infection isolates and ca. 50 to 60% of isolates from patients with recurrent or complicated infections (10, 26).

The currently recommended empirical antimicrobial regimen for treating acute uncomplicated bacterial cystitis in otherwise-healthy adult nonpregnant females is a 3-day course of double-strength trimethoprim-sulfamethoxazole (SXT) in settings where the prevalence of SXT resistance is less than 10 to 20% (7, 33). Alternative therapy for uncomplicated urinary infections in settings with more than 10 to 20% SXT resistance may include a fluoroquinolone, nitrofurantoin, or fosfomycin (33). The Infectious Diseases Society of America (IDSA) also recommends that physicians obtain information on local resistance rates and that ongoing local, regional, and national surveillance be conducted to monitor changes in susceptibility of uropathogens and the suitability of empirical therapy recommendations (33). Surveillance at the institutional level may be particularly important since previous studies have shown that the activity of SXT against urinary isolates of *E. coli* can vary considerably by geographic location (11, 29).

A national surveillance initiative specifically describing antimicrobial resistance among common urinary pathogens in the United States or Canada has not published data for more than 5 years (10–13, 15–17, 22, 29, 35, 36). To address this need, the present study reports on the *in vitro* activities of SXT, ciprofloxacin, nitrofurantoin, and other comparator agents evaluated against a recent Canadian collection of pathogens isolated from outpatients and inpatients with urinary tract infections in the period from 2007 to 2009.

MATERIALS AND METHODS

Bacterial isolate collection. From January 2007 to December 2009 tertiary-care medical centers in eight of 10 Canadian provinces (12 centers in 2007; 10 centers in 2008; 15 centers in 2009) submitted bacterial pathogens isolated from midstream and catheter urine specimens to a coordinating laboratory (Health Sciences Centre, Winnipeg, Manitoba, Canada); 10 medical centers participated in all 3 years of the study. Isolate collection was part of the ongoing CANWARD

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surveillance study and involved outpatients attending hospital clinics and emergency rooms, as well as inpatients present on medical and surgical wards and in intensive care units (34). Each study site was asked to submit only clinically significant isolates, as defined by local site criteria, from outpatients and inpatients with urinary, respiratory, wound, and bloodstream infections (34). Fifty (in 2009) to one-hundred (in 2007 and 2008) consecutive urinary tract isolates per year per medical center site (one isolate per patient) were collected. Primary isolate identification was performed by the submitting medical center site and confirmed by the coordinating laboratory, as required, based on morphological characteristics and spot tests (4). If an isolate identification made by the coordinating laboratory did not match that provided by the submitting site, the isolate was removed from the study. The CANWARD surveillance study collected and tested a total of 7,229 isolates in 2007, 4,590 isolates in 2008, and 4,550 isolates in 2009; of these, 1,211 (16.8%; 2007), 983 (21.4%; 2008), and 749 (16.5%; 2009) were urinary tract isolates.

Antimicrobial susceptibility testing. The *in vitro* activities of antimicrobials were determined by using in-house prepared 96-well broth microdilution panels in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (5) with MICs interpreted according to CLSI M100-S20 (2010) breakpoints (3). Yeasts, nonspeciated coagulase-negative staphylococci, *Streptococcus agalactiae*, viridans streptococci, *Moraxella catarrhalis*, and species with fewer than 10 isolates were not tested for antimicrobial susceptibilities.

ESBL screening and confirmation. Screening for production of extended-spectrum β-lactamases (ESBLs) by isolates of *E. coli*, *Klebsiella* spp., and *Proteus mirabilis* was performed as recommended by CLSI (3). Confirmatory testing used the CLSI disk diffusion method (18) with disks containing ceftazidime (30 μg), ceftazidime-clavulanic acid (30 μg/10 μg), cefotaxime (30 μg), and cefotaxime-clavulanic acid (30 μg/10 μg) supplied by Mast Diagnostics (United Kingdom).

Methicillin-resistant *Staphylococcus aureus* (MRSA) confirmation. The potential methicillin resistance in *S. aureus* isolates was screened for using the cefoxitin disk test described by the CLSI (3) and confirmed by PCR amplification of the *mecA* gene (24); staphylococcal protein A (*spa*) typing (8) was used to assess whether the isolates were community associated or healthcare associated.

VRE confirmation. Potential vancomycin resistance in *Enterococcus faecium* and *Enterococcus faecalis* (VRE) isolates was confirmed with the vancomycin agar dilution test as described by CLSI (3). All confirmed VRE isolates underwent a multiplex PCR method to detect the presence of *vanA*, *vanB*, *vanC*, *vanD*, or *vanE* (2).

Statistical methods. Statistical analysis of antimicrobial susceptibility testing results and patient demographic data were undertaken for *E. coli*; other pathogens were not analyzed due to their low numbers. Isolates of *E. coli* with intermediate range MICs were included in the resistant group for analyses. A nominal logistic regression model was used to analyze temporal trends in resistance to the tested antimicrobials over the three study years. To determine whether patient variables were independently associated with antimicrobial resistance to specific agents, we initially performed a univariate analysis of resistance and patient variables. Variables associated with a P value of <0.1 were included in a second degree factorial multivariate logistic regression model to determine potential interactions between gender, age, hospital location, and geographic location. Hospital location was defined as either outpatient (emergency departments or outpatient clinics) or inpatient (medical, surgical, or intensive care unit wards). A P value of ≤ 0.05 was considered statistically significant. All statistical analysis was done by using JMP version 9.0 (SAS, Cary, NC).

RESULTS

The most frequently isolated urinary pathogens tested in the CANWARD 2007–2009 study (n = 2,943) were *E. coli* (54%), enterococci (14%), *K. pneumoniae* (9%), *P. mirabilis* (4%), *P. aeruginosa* (3%), and *S. aureus* (3%). These six pathogens accounted for almost 90% of all urinary tract isolates collected in the CANWARD study (Table 1). The annual rank order of the six most common urinary pathogens (*E. coli*, enterococci, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*) was consistent for 2007, 2008, and 2009 (data not shown). Differences were noted in the composition of pathogens isolated from outpatients (emergency departments and outpatient clinics) and inpatients (medical, surgical, and intensive care unit wards). Among outpatients (n = 1,506), the most common pathogens were *E. coli* (64%), *Enterococcus* spp. (10%), *K. pneumoniae* (7%), *P. mirabilis* (3%), *Streptococcus agalactiae* (3%), *S. aureus* (2%), and *P. aeruginosa* (2%). Among inpatients (n = 1,437) the most common pathogens were *E. coli* (43%), *Enterococcus* spp. (18%), *K. pneumoniae* (12%), *P. aeruginosa* (5%), *P. mirabilis* (5%), *S. aureus* (3%), *Enterobacter cloacae* (3%), and *Klebsiella oxytoca* (2%).

TABLE 1. Most common pathogens isolated from urine in the CANWARD Surveillance Program, 2007 to 2009

Rank	Pathogen	% (no.) of 2,943 urine isolates collected in 2007 to 2009[a]
1	*Escherichia coli*	53.7 (1,581)
2	*Enterococcus* spp.	13.9 (410)
3	*Klebsiella pneumoniae*	9.3 (274)
4	*Proteus mirabilis*	4.1 (122)
5	*Pseudomonas aeruginosa*	3.4 (100)
6	*Staphylococcus aureus*	2.7 (80)
7	*Enterobacter cloacae*	1.8 (54)
8	*Streptococcus agalactiae*	1.8 (52)
9	*Klebsiella oxytoca*	1.6 (46)
10	*Citrobacter freundii*	1.0 (29)
11	*Morganella morganii*	0.8 (24)
12	*Staphylococcus saprophyticus*	0.5 (14)
13	*Citrobacter koseri*	0.5 (14)
14	*Serratia marcescens*	0.4 (12)
15	*Enterobacter aerogenes*	0.3 (10)
–[b]	Others	4.1 (121)

[a] The collection of 2,943 isolates contained 1,211 isolates from 2007, 983 isolates from 2008, and 749 isolates from 2009. The annual rank order of the six most common urinary pathogens (*E. coli*, enterococci, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*) was consistent for 2007, 2008, and 2009 (data not shown).

[b] The "Others" pathogen category was composed of 58 isolates of coagulase-negative *Staphylococcus* spp. and 63 isolates from 27 other species.

The rates of susceptibility to SXT were 78, 86, 84, and 93%, respectively, for *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. aureus*; enterococci and *P. aeruginosa* are intrinsically resistant to SXT (Table 2). The rates of susceptibility to nitrofurantoin were 96, 97, 33, and 100%, respectively, for *E. coli*, enterococci, *K. pneumoniae*, and *S. aureus*; *P. mirabilis* and *P. aeruginosa* are intrinsically resistant to nitrofurantoin. The rates of susceptibility to ciprofloxacin were 81, 40, 86, 81, 66, and 41%, respectively, for *E. coli*, enterococci, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus*. Statistical analysis of the resistance rates (resistant plus intermediate isolates) by year for *E. coli* over the 3-year study period demonstrated that increased resistance rates occurred only for amoxicillin-clavulanate (from 1.8 to 6.6%; $P < 0.001$) and SXT (from 18.6 to 24.3%; P = 0.02) (data not shown).

The ESBL rates for *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis* were 3% (52/1,581), 3% (8/274), 0% (0/46), and 0% (0/122), respectively. Four vancomycin-resistant isolates (1% [4/410]; all *vanA* positive) of enterococci were identified. Thirty-five isolates of *S. aureus* were found to be methicillin resistant (MRSA); 33 were healthcare-associated MRSA *spa* types (one of the 10 Canadian MRSA epidemic *spa* types excluding CMRSA7 [USA400] and CMRSA10 [USA300]), one was a community-associated MRSA *spa* type (CMRSA10 [USA300]), and one was a unique MRSA *spa* type (not one of the 10 Canadian MRSA epidemic types). Eleven of the 35

TABLE 2. *In vitro* activities of antimicrobial agents against the most common pathogens isolated from urine in the CANWARD 2007–2009 study

Organism (no. tested) and antimicrobial agent[a]	MIC (μg/ml)			%[b]		
	MIC_{50}	MIC_{90}	Range	Susceptible	Intermediate	Resistant
Escherichia coli (1,581)						
Amoxicillin-clavulanate*	4	8	≤0.06–>32	95.9	3.2	0.9
Ceftazidime	≤0.5	1	≤0.5–>32	96.5	0.3	3.2
Ceftriaxone	≤1	≤1	≤1–>64	95.3	0.3	4.4
Ciprofloxacin*	≤0.06	>16	≤0.06–>16	80.5	0.4	19.1
Gentamicin	≤0.5	2	≤0.5–>32	92.2	0.3	7.5
Meropenem	≤0.12	≤0.12	≤0.12	100	0	0
Nitrofurantoin*	16	32	≤0.5–>256	95.9	2.2	1.9
Piperacillin-tazobactam	2	4	≤1–>512	97.7	1.4	0.9
Trimethoprim-sulfamethoxazole*	≤0.12	>8	≤0.12–>8	77.9	NA	22.1
Enterococcus spp. (410)[c]						
Ampicillin	0.5	1	≤0.06–>32	94.5	NA	5.5
Ciprofloxacin	2	>16	≤0.06–>16	39.9	21.1	39.1
Nitrofurantoin	8	8	≤0.5–256	97.4	0.7	2.0
Vancomycin	1	2	≤0.25–>32	99.0	0	1.0
Klebsiella pneumoniae (274)						
Amoxicillin-clavulanate	2	8	0.5–16	97.3	2.7	0
Ceftazidime	≤0.5	1	≤0.5–>32	96.9	1.2	1.9
Ceftriaxone	≤1	≤1	≤1–>64	91.9	1.5	6.6
Ciprofloxacin	≤0.06	4	≤0.06–>16	85.7	2.2	12.1
Gentamicin	≤0.5	≤0.5	≤0.5–>32	94.5	0.4	5.1
Meropenem	≤0.12	≤0.12	≤0.12–0.25	100	0	0
Nitrofurantoin	64	256	8–>256	32.8	22.1	45.1
Piperacillin-tazobactam	2	16	≤1–512	95.6	2.2	2.2
Trimethoprim-sulfamethoxazole	≤0.12	>8	≤0.12–>8	86.1	NA	13.9
Proteus mirabilis (122)						
Amoxicillin-clavulanate	1	8	0.5–>32	94.8	2.6	2.6
Ceftazidime	≤0.5	≤0.5	≤0.5–4	100	0	0
Ceftriaxone	≤1	≤1	≤1–4	97.5	0.8	1.6
Ciprofloxacin	≤0.06	2	≤0.06–>16	81.2	10.7	8.2
Gentamicin	1	2	≤0.5–>32	96.7	1.6	1.6
Meropenem	≤0.12	≤0.12	≤0.12–0.25	100	0	0
Nitrofurantoin	128	256	64–256	0	6.0	94.0
Piperacillin-tazobactam	≤1	≤1	≤1–8	100	0	0
Trimethoprim-sulfamethoxazole	≤0.12	>8	≤0.12–>8	84.4	NA	15.6
Pseudomonas aeruginosa (100)						
Ceftazidime	4	8	1–>32	93.0	1.8	5.3
Ceftriaxone	32	>64	4–>64	16.0	46.0	38.0
Ciprofloxacin	0.5	>16	≤0.06–>16	66.0	7.0	27.0
Gentamicin	4	16	≤0.5–>32	76.0	10.0	14.0
Meropenem	0.5	4	≤0.12–>32	91.0	2.0	7.0
Piperacillin-tazobactam	4	64	2–512	92.0	NA	8.0
Staphylococcus aureus (80)[d]						
Amoxicillin-clavulanate	1	32	0.12–32	57.5	NA	42.6
Ceftazidime	16	>32	8–>32	23.3	30.2	46.5
Ceftriaxone	4	>64	1–>64	57.5	7.5	35.0
Ciprofloxacin	>16	>16	0.12–>16	41.3	0	58.8
Gentamicin	≤0.5	2	≤0.5–>32	90.0	0	10.0
Nitrofurantoin	16	16	8–32	100	0	0
Meropenem	0.12	16	≤0.12–>32	80.0	6.3	13.8
Piperacillin-tazobactam	≤1	128	≤1–>512	62.5	NA	37.5
Trimethoprim-sulfamethoxazole	≤0.12	≤0.12	≤0.12–>8	92.5	NA	7.5
Vancomycin	1	1	≤0.25–1	100	0	0
Enterobacter cloacae (54)						
Amoxicillin-clavulanate	16	>32	2–>32	31.4	31.4	37.1
Ceftazidime	≤0.5	8	≤0.5–>32	88.6	2.9	8.6
Ceftriaxone	≤1	32	≤1–>64	75.9	3.7	20.4
Ciprofloxacin	≤0.06	0.12	≤0.06–4	94.4	1.9	3.7
Gentamicin	≤0.5	≤0.5	≤0.5–>32	98.2	0	1.9

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TABLE 2—*Continued*

Organism (no. tested) and antimicrobial agent[a]	MIC (μg/ml)			% [b]		
	MIC_{50}	MIC_{90}	Range	Susceptible	Intermediate	Resistant
Meropenem	≤0.12	≤0.12	≤0.12–0.5	100	0	0
Nitrofurantoin	32	128	16–128	50.0	36.4	13.6
Piperacillin-tazobactam	2	16	≤1–256	90.7	5.6	3.7
Trimethoprim-sulfamethoxazole	≤0.12	1	≤0.12–>8	92.6	NA	7.4
Klebsiella oxytoca (46)						
Amoxicillin-clavulanate	2	8	1–16	90.0	10.0	0
Ceftazidime	≤0.5	0.5	≤0.5–1	100	0	0
Ceftriaxone	≤1	4	≤1–16	84.8	2.2	13.0
Ciprofloxacin	≤0.06	≤0.06	≤0.06–4	97.8	0	2.2
Gentamicin	≤0.5	≤0.5	≤0.5–8	97.8	2.2	0
Meropenem	≤0.12	≤0.12	≤0.12–0.12	100	0	0
Nitrofurantoin	16	32	4–64	95.0	5.0	0
Piperacillin-tazobactam	2	256	≤1–>512	80.4	2.2	17.4
Trimethoprim-sulfamethoxazole	≤0.12	≤0.12	≤0.12–>8	97.8	NA	2.2
Citrobacter sp. (29)						
Amoxicillin-clavulanate	8	32	1–>32	52.9	29.4	17.7
Ceftazidime	0.5	>32	≤0.5–>32	87.5	0	12.5
Ceftriaxone	≤1	32	≤1–64	86.2	0	13.8
Ciprofloxacin	≤0.06	4	≤0.06–>16	86.2	3.5	10.3
Gentamicin	≤0.5	4	≤0.5–32	93.1	3.5	3.5
Meropenem	≤0.12	≤0.12	≤0.12	100	0	0
Nitrofurantoin	16	32	16–128	91.7	0	8.3
Piperacillin-tazobactam	2	64	≤1–64	89.7	10.3	0
Trimethoprim-sulfamethoxazole	≤0.12	>8	≤0.12–>8	86.2	NA	13.8
Morganella morganii (24)						
Amoxicillin-clavulanate	32	>32	1–>32	11.8	29.4	58.8
Ceftazidime	≤0.5	0.5	≤0.5–1	100	0	0
Ceftriaxone	≤1	≤1	≤1–4	95.8	0	4.2
Ciprofloxacin	≤0.06	4	≤0.06–>16	83.3	4.2	12.5
Gentamicin	≤0.5	>32	≤0.5–>32	83.8	0	16.7
Meropenem	≤0.12	≤0.12	≤0.12–0.25	100	0	0
Nitrofurantoin	64	64	32–64	9.1	90.9	0
Piperacillin-tazobactam	≤1	≤1	≤1–32	95.8	4.2	0
Trimethoprim-sulfamethoxazole	0.25	>8	≤0.12–>8	70.8	NA	29.2

[a] The ESBL rates for *E. coli*, *K. pneumoniae*, *K. oxytoca,* and *P. mirabilis* were 3.3% (52/1,581), 2.9% (8/274), 0% (0/46), and 0% (0/122), respectively. *, There was no significant change in the percent susceptibility for *E. coli* by year (2007, 2008, and 2009; $P > 0.05$).

[b] NA, not available.

[c] The number includes four vancomycin-resistant isolates (all *vanA* positive).

[d] The β-lactam MICs were interpreted according to CLSI breakpoints and not corrected to resistant for isolates of MRSA. A total of 35 isolates were determined to be methicillin-resistant *S. aureus* (MRSA); 33 were healthcare-associated MRSA *spa* types (one of the 10 Canadian MRSA epidemic *spa* types excluding CMRSA7 [USA400] and CMRSA10 [USA300]), one was a community-associated MRSA *spa* type (CMRSA7 [USA400] or CMRSA10 [USA300]), and one was a unique MRSA *spa* type (not one of the 10 Canadian MRSA epidemic types) (8).

MRSA isolates were from outpatients (nine were healthcare-associated MRSA *spa* types, one was a community-associated MRSA *spa* type, and one was a unique MRSA *spa* type), and the remaining 24 isolates were from inpatients (all were healthcare-associated MRSA *spa* types).

For isolates of *E. coli*, in a multivariate logistic regression model, hospital location was independently associated with resistance to ceftazidime ($P = 0.002$; data not shown), ceftriaxone ($P = 0.029$; data not shown), and ciprofloxacin ($P = 0.026$), with higher rates of resistance observed in inpatient areas (medical, surgical, and intensive care unit wards) (Table 3). Increased age was associated with resistance to ciprofloxacin ($P < 0.001$) and gentamicin ($P = 0.021$) (data not shown) and with resistance to two or more commonly prescribed oral agents (amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and SXT) ($P = 0.005$). For isolates of *E. coli*, 193 of 1,581 (12.2%) were resistant to two or more frequently prescribed antimicrobial agents; 94.8% (183/193) and 91.7% (177/193) of coresistant isolates were resistant to ciprofloxacin and SXT, respectively, while resistance to amoxicillin-clavulanate (15.5%, 30/193) and nitrofurantoin (6.7%, 13/193) were much less frequently associated with coresistant isolates (Table 4). Male gender was associated with increased rates of resistance to nitrofurantoin ($P = 0.004$) and SXT ($P = 0.037$). No other statistically significant associations were observed.

DISCUSSION

The most recent Canadian national surveillance study to publish data on antimicrobial susceptibilities of urinary tract pathogens was on isolates collected by 10 centers in 2003 to 2004 (35). In that study, 496 outpatient urinary tract isolates were tested, of which 56.9% were *E. coli*, 11.4% were *K. pneumoniae*, 7.8% were *Enterococcus* spp., 4.9% were *P. mirabilis*,

TABLE 3. Demographic characteristics of patients from whom *E. coli* was isolated from urine during the CANWARD 2007–2009 study

Patient category	No. of isolates	% Isolates resistant[a] to:			
		AMC	CIP	NIT	SXT
Gender					
Female	1,224	4.3	17.2	2.9	20.8
Male	357	3.3	27.2	7.9	26.3
Age (yr)					
<18	241	4.7	3.7	2.4	20.7
18–64	673	3.0	14.7	2.1	21.8
>64	667	5.1	30.0	6.5	22.8
Hospital location					
Clinic/office	456	5.2	15.8	3.6	23.5
Emergency room	501	2.8	12.4	3.3	19.0
Intensive care unit	45	7.7	17.8	5.9	24.4
Medical ward	479	3.5	27.6	4.4	24.0
Surgical ward	100	10.5	34.0	8.3	21.0
Geographic location[b]					
Western Canada	527	5.2	23.5	3.6	23.0
Ontario	529	2.3	19.5	4.4	21.9
Eastern Canada	525	4.5	15.4	4.3	21.3

[a] For ciprofloxacin (CIP), nitrofurantoin (NIT), and amoxicillin-clavulanate (AMC), intermediate isolates were added to resistant isolates and are reported as resistant for this analysis. SXT, trimethoprim-sulfamethoxazole.

[b] Western Canada includes the provinces of British Columbia, Alberta, Saskatchewan, and Manitoba; eastern Canada includes Quebec, Nova Scotia, and New Brunswick.

TABLE 4. Coresistant phenotypes among 1,581 *E. coli* urinary isolates tested against amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and trimethoprim-sulfamethoxazole in 2007 to 2009[a]

Coresistant phenotype	No. of coresistant isolates	%	
		Coresistant isolates	All isolates
CIP, SXT	150	77.7	9.5
AMC, CIP	13	6.7	0.8
AMC, CIP, SXT	9	4.7	0.6
AMC, SXT	8	4.1	0.5
CIP, NIT, SXT	8	4.1	0.5
CIP, NIT	3	1.6	0.2
NIT, SXT	2	1.0	0.1
Total	193	100	12.2

[a] For amoxicillin-clavulanate (AMC), ciprofloxacin (CIP), and nitrofurantoin (NIT), intermediate isolates were added to resistant isolates and are reported as resistant for this analysis. SXT, trimethoprim-sulfamethoxazole. A total of 67.6% (1,069/1,581) of isolates were uniformly susceptible to AMC, CIP, NIT, and SXT; 20.2% (319/1,581) of the isolates were intermediate or resistant to AMC, CIP, NIT, or SXT of the 193 coresistant isolates. A total of 103 (53.4%) were from outpatients, and 90 (46.6%) were from inpatients.

2.7% were *S. aureus*, and 1.1% were *P. aeruginosa*. The resistance rates for *E. coli* in that study were as follows: nitrofurantoin, 0%; ciprofloxacin, 1.1%; and SXT, 17.7% (35). A similar Canadian study of outpatient urinary tract isolates conducted in 1998, using most of the same centers, reported that 84.1% of the isolates were *E. coli*, 3.8% were *K. pneumoniae*, 2.8% were *Enterococcus* spp., 2.6% were *P. mirabilis*, 0.7% were *P. aeruginosa*, and 0.4% were *S. aureus* (36). The resistance rates for *E. coli* ($n = 1,681$) in that study were as follows: nitrofurantoin, 0.1%; ciprofloxacin, 1.2%; and SXT, 18.9% (36). In comparison, the present study found resistance rates of 1.9, 19.1, and 22.1%, respectively, to nitrofurantoin, ciprofloxacin, and SXT among the 1,581 isolates of *E. coli* tested (Table 2). Resistance to ciprofloxacin (~16-fold increase) and nitrofurantoin (19-fold increase) have both shown greater relative increases compared to SXT resistance, which has remained relatively steady from 1998 (17.7%) to 2007 to 2009 (19.1%). In previous United States-based studies, SXT resistance increased from 7 to 9% in 1989 to 1992 (11, 13, 23, 32) to 17 to 18% in 1995 to 1999 (11–13, 16). Fluoroquinolone resistance was ≤1% in each of the aforementioned studies and nitrofurantoin resistance was reported to be ≤2% on national and regional levels in the United States. The 1998 SENTRY surveillance program, reporting on isolates of *E. coli* collected from 26 U.S. centers, found the overall prevalence of SXT resistance to be 23.3% (22), a level very similar to the resistance rate found a year earlier (25.2%) by the same surveillance program (15).

In our study, the only oral agents with reliable *in vitro* activity against *E. coli* were nitrofurantoin (used for cystitis only) and amoxicillin-clavulanate; however, amoxicillin-clavulanate resistance increased significantly from 1.8% in 2007 to 6.6% in 2009 ($P < 0.001$). The emergence of ESBLs, in addition to high rates of fluoroquinolone resistance in all inpatient and outpatient Gram-negative isolates (1, 25, 28), has been identified by others as a cause for concern. The decreased susceptibility to fluoroquinolones, amoxicillin-clavulanate, and nitrofurantoin may reflect ongoing antimicrobial selective pressure. Fluoroquinolone resistance occurs predominantly in isolates resistant to other agents and less commonly alone, as previously reported (17–19, 29). Although amoxicillin-clavulanate is considered a second-line agent for urinary tract infection, the increasing prevalence of resistance to SXT and fluoroquinolones in *E. coli* (9) may be driving increased reliance on this agent for the management of cystitis.

Using multivariate analysis, a number of patient demographic factors were found to be associated with antimicrobial resistance in *E. coli* isolates (Table 3). The most reliably associated factor was an inpatient setting with significantly higher rates of resistance to ciprofloxacin observed in inpatient areas (medical, surgical, and intensive care unit wards); amoxicillin-clavulanate, nitrofurantoin, and SXT did not show this association. This observation was not unexpected and may be a result of antimicrobial selective pressure in inpatient settings (1, 30). Increased age was associated with resistance to ciprofloxacin ($P < 0.001$) and with resistance to two or more commonly prescribed oral agents (amoxicillin-clavulanate, ciprofloxacin, nitrofurantoin, and SXT) ($P = 0.005$). In all cases, the direction of the association was one of decreased susceptibility (or increased multidrug resistance [MDR]) with increasing age. This has been reported previously and may reflect increasing cumulative exposure to antimicrobial agents, increasing cumulative exposure to healthcare settings, more comorbidities, and increased durations of hospital stays (1, 30). Increasing cumulative exposure is particularly relevant to fluoroquinolones, which are used in large quantities in both hospital and outpatient settings.

Among isolates of *E. coli*, 193 of 1,581 (12.2%) were resistant to two or more frequently prescribed antimicrobial agents; 94.8% (183/193) and 91.7% (177/193) of coresistant isolates were resistant to ciprofloxacin and SXT, respectively, while resistance to amoxicillin-clavulanate (15.5%, 30/193) and nitrofurantoin (6.7%, 13/193) were much less frequently associated with coresistant isolates (Table 4). Male gender was associated with increased rates of resistance to nitrofurantoin ($P = 0.004$) and SXT ($P = 0.037$). Males are known to experience more complicated urinary tract infections than women, requiring longer durations of treatment, which may play a significant role in the development of isolates resistant to agents commonly used for urinary tract infections. Gender was associated with resistance to some antimicrobials tested, notably fluoroquinolones, nitrofurantoin, and gentamicin. Although a complete explanation for this finding is elusive, it has been observed in a number of other settings (1, 6, 30). Possible explanations include unmeasured confounding variables such as hospital length of stay and comorbidities.

A possible explanation for increasing antimicrobial resistance among uropathogenic isolates of *E. coli* may involve clonal spread of resistant isolates (14, 18, 21, 27). Johnson et al. (14) reported in 2009 that clonal groups of *E. coli*, particularly clonal group A, O15:K52:H1, and ST131 (may also harbor CTX-M-15, an ESBL), are major contributors (accounting for ca. 50% of SXT-resistant and fluoroquinolone-resistant urine isolates from Canadian patients) to the spread of SXT and fluoroquinolone resistance in Canadian urinary tract isolates. These clonal groups are also known to be more virulent than other resistant isolates, and this may possibly have contributed to their success as emerging MDR pathogens. Other authors studying Canadian urinary tract isolates have reported similar findings supporting the notion of clonal spread of antimicrobial resistant uropathogenic *E. coli* (18, 21, 27). Infection control strategies to define and interrupt transmission pathways, as well as reductions in selective pressure by limiting antimicrobial use, are currently nonexistent (14). The O15:K52:H1 clonal group originally accounted for an outbreak of SXT-resistant urinary tract infections and extra-intestinal infections in South London in 1986 and 1987 and has since acquired fluoroquinolone resistance (14). Clonal expansion and spread of MDR isolates may potentially occur when these isolates are selected for by administration of any antimicrobial agent to which the isolate harbors resistance in patients colonized or infected with such an isolate. MDR isolates may complicate the therapeutic management of patients with infection by increasing morbidity and treatment costs and by limiting therapeutic options (18). The observation that fluoroquinolone resistance among *E. coli* and other Gram-negative bacilli is predominantly found among MDR isolates suggests that fluoroquinolone resistance will be maintained and perhaps increase further even if other antimicrobial classes are used to treat infections arising from these pathogens due to coselection (17).

The limitations of the present study include the absence of clinical and outcome data describing the types and severity of the urinary tract infections from which the isolates were derived. The strengths include the large sample size used and the broadly distributed and systematically collected isolates. It must also be recognized that traditional surveillance data, such as those studied here and elsewhere, may bias toward an overreporting of resistance in patients with acute uncomplicated cystitis because the treatment of acute cystitis in otherwise healthy adult females is empirical. Therefore, isolates tested for antimicrobial susceptibility may be predominantly from patients for whom previous antimicrobial treatment failed or from patients with other underlying risk factors. There may also have been a decrease in physician orders for routine urine cultures over time. It is recognized that performing patient-based studies would be an optimal alternative but would be costly and likely not practical for regional and national data assimilation (17).

In conclusion, *E. coli* remains a common pathogen isolated from urine samples submitted to clinical microbiology laboratories for culture, although it is less frequently isolated from inpatient (43%) than outpatient (64%) samples and appears to be less common among outpatients samples than it was in a similar 2000 study (84.1% *E. coli*) involving the same group of hospitals (36). In the present study, *E. coli*, enterococci, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. aureus* accounted for almost 90% of the isolates. In both hospital and outpatient settings, resistance, particularly to traditional first-line agents such as fluoroquinolones and SXT, has increased substantially across Canada. A number of factors, most importantly inpatient status, are associated with resistance to antimicrobials and MDR, and these factors need to be taken into consideration when empirical treatment is prescribed. Ongoing surveillance is required to monitor developments in resistance in *E. coli*, and the development of antimicrobials for drug-resistant Gram-negative organisms is needed (20). The importance of recognizing and defining the extent of antimicrobial resistance in urinary tract pathogens and identifying differences in prevalence and resistance patterns in these pathogens should be a priority.

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